

A Colorful New Way to Look at the Nuclear Pore!

Stephen W. Carmichael,¹ Mayo Clinic

Light microscopy has many advantages and several disadvantages. One of the advantages is that different wavelengths are perceived as different colors, and with the proper use of dyes, filters, etc. several different structures can be imaged in the same object. One the major disadvantages is that the resolution is limited by the wavelength, a limit known as the diffraction barrier. Several recently-developed techniques have allowed light microscopy to "break" the diffraction barrier, a phenomenon known as "super-resolution." Lothar Schermelleh, Peter Carlton, Sebastian Haase, Lin Shao, Lukman Winoto, Peter Kner, Brian Burke, Cristina Cardoso, David Agard, Mats Gustafsson, Heinrich Leonhardt, and John Sedat have created a new microscope that not only achieves super-resolution, but creates images in 3 dimensions and multiple colors.² Not only that, but preparing specimens to examine with this microscope uses conventional methods, and they claim the microscope is easy to use!

This is not to mean the microscope is simple. It is not. The basic principle is comparable to a Moiré pattern created when two translucent objects are inserted into the same optical path and the resulting pattern contains additional information about the structure of those objects. The microscope platform designed and constructed by Sedat, Agard, and their group was named "OMX" (Optical Microscope, eXperimental). To operate it for 3-dimensional structured illumination microscopy (3D-SIM), laser illumination is passed through a diffraction grating. The grating is mounted on a high-precision piezoelectric stage that provides phase control of the pattern by translating the grating into five positions. In turn, this stage is mounted on a rotational stage that orients the grating into three different angular orientations. The incident light is diffracted into multiple orders, the innermost three of which are focused at the back focal plane of the 100X objective lens. These three beams intersect and interfere in the sample plane, producing an illumination pattern in 3D. Emit-

1. The author gratefully acknowledges Dr. John Sedat for reviewing this article.
2. Schermelleh, L., P.M. Carlton, S. Haase, L. Shao, L. Winoto, P. Kner, B. Burke, M.C. Cardoso, D.A. Agard, M.G.L. Gustafsson, H. Leonhardt, and J.W. Sedat, Subdiffraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy, *Science* 320:1332-1336, 2008.

ted light from the sample passes through a set of four dichroic mirrors (not filters, as is done most commonly) that direct light of different wavelengths into four independently-controlled CCD cameras. All of the operations of the microscope (only of few of which are described here) are controlled by custom-written software.

Schermelleh, Carlton *et al.* used 3D-SIM to probe higher-order chromatin structure and the relative localization of nuclear pores, the nuclear lamina, and chromatin at the periphery of the nucleus of cultured muscle cells of mice. In vertebrates, the nuclear pore complex (NPC) is a highly ordered assembly of proteins that mediates communication and selective exchange between the nucleoplasm and the cytoplasm. The structure of the NPC has been well characterized by electron microscopy but this technique does not provide an overall 3D view of the entire nucleus with specific labeling of individual proteins. 3D-SIM can bridge the gap between conventional forms of light microscopy and electron microscopy.

They first examined nuclei in cells stained with 4,6-diamidino-2-phenylindole (DAPI) and found that chromatin has a fibrous substructure. Unexpectedly, 3D-SIM images showed thousands of well-defined holes in the DAPI staining. The size, number, and position of these holes suggested they represent exclusion of DNA from NPCs. To test this, Schermelleh, Carlton *et al.* co-immunostained the cells with NPC-specific antibodies and antibodies against lamin B (lamin B is a major component of the nuclear lamina that lines and stabilizes the nuclear envelope). They consistently observed the peripheral heterochromatin rim outlined by a fine heterogeneous layer of the nuclear lamina and nuclear pore signals further above the lamina. For comparison, they also used an antibody against a protein known to be part of the nucleoplasmic side of the NPC and obtained a pore signal in the same plane as the lamin B signal. Almost every DAPI void contained a focus of NPC staining and *vice versa*, suggesting that most if not all NPCs exclude chromatin from their immediate vicinity.

Schermelleh, Carlton *et al.* also performed these experiments using state-of-the-art confocal laser scanning microscopes. The features they detected by 3D-SIM could not be resolved. 3D-SIM is currently the only super-resolution instrument that can produce multicolor 3D images of whole cells. Interestingly, these results were obtained with standard cytological methods. This gives us a colorful new way to look at sub-cellular structures! ■

INDEX OF ARTICLES

A Colorful New Way to Look at the Nuclear Pore!3
Stephen W. Carmichael, Mayo Clinic

On the Sub-Nanometer Resolution of Scanning Electron and Helium Ion Microscopes6
András E. Vladár, Michael T. Postek, and Bin Ming Nat'l. Inst. of Standards and Technology, Gaithersburg, MD

A Versatile and Affordable Plunge Freezing Instrument for Preparing Frozen Hydrated Specimens for Cryo Transmission Electron Microscopy (CryoEM) 14
Linda Melanson, Gatan, Inc., Pleasanton, CA

Atomic Layer Deposition and Vapor Deposited SAMS in a CrossBeam FIB-SEM Platform: A Path To Advanced Materials Synthesis18
E. L. Principe†, C. Hartfield‡, R. Kruger‡, A. Smith‡, R. Dubois, K. Scammon**, B. Kempshall*** †Carl Zeiss SMT, Inc.‡ Omniprobe, Inc. *, Colonial Metals, Inc. **, University of Central Florida, NanoSpectre****

Pushing the Envelope in Atomic Force Microscopy26
M. G. Heaton & J. P. Cleveland, Asylum Research, Santa Barbara, CA

Dip Pen Nanolithography: A Desktop Nanofabrication Approach Using High-Throughput Flexible Nanopatterning30
Jason Haaheim and Omkar A. Nafday, NanoInk Inc.

A Compact Field Emission SEM for Low Voltage Imaging .. 34
Jim Rynne, Novelx, Inc., Lafayette, CA

TEM Sample Preparation: An Interdisciplinary Website 38
J. Ayache¹, L. Beaudier², J. Boumendil³, G. Ehret⁴ and D. Laub⁵, ¹CNRS-UMR, Villejuif, France; ²CNRS-UPR, Paris; ³U.Claude Bernard-Lyon, Villeurbanne, France; ⁴Institut Physique et Chimie des Matériaux de Strasbourg, France; ⁵EPFL-CIME, Lausanne, Switzerland

Microparticles/Exosomes: Isolation and TEM Analysis 42
Natalie Bauer, Jyoti Rai, Hairu Chen, Lillianne Harris, Lalita Shevde, Tim Moore, and Judy King, U. of South Alabama, Mobile, AL

New Ultra-Thin Pure Silicon Window Grids for Transmission Electron Microscopy Samples 46
James Roussie, TEMwindows.com, Rochester, NY

Preparing Biological Samples for Analysis by High Vacuum Techniques 48
S.G. Ostrowski, T.L. Paxon, L. Denault, KP. McEvoy, and V.S. Smentkowski, General Electric Global Research Ctr., Niskayuna, NY

Pioneers in Optics: Friedrich Johann Karl Becke and Anders Jöns Ångström 54
Michael W. Davidson, National High Magnetic Field Laboratory, The Florida State University, Tallahassee, FL

Microscopy-101: Glass Knives vs. Diamond Knives56
Lou Ann Miller, Center for Microscopic Imaging, Veterinary Medicine, University of Illinois

Industry News58

NetNotes60

Advertiser's Index78

ABOUT THE COVER

This image illustrating pitting on softwood fibers in a 15-µm thick section of White Pine Wood (*P. strobus*) was taken by Namitha R. Sundara of Buckeye Technologies, Inc. in Memphis, Tennessee. The image was captured in polarized light on an Olympus BX51 light microscope at an original magnification of 100x. The camera used to capture the image was an Olympus Soft Imaging System Colorview III Digital Microscope Camera.

COMING EVENTS

2009

- ✓ **Focus On Microscopy 2009**
April 5-8, 2009, Krakow, Poland
www.FocusOnMicroscopy.org
- ✓ **EELS and EFTEM Analysis Course**
April 6-9, 2009, Pleasanton, CA
www.gatan.com
- ✓ **2009 MRS Spring meeting**
April 13-17, 2009, San Francisco, CA
www.mrs.org
See especially Symposium JJ on Nanoscale Electromechanics and Piezoresponse, www.mrs.org/s_mrs/sec.asp?CID=14465&DID=211517 Force Microscopy
- ✓ **American Soc. for Biochemistry and Molecular Biology**
April 18-22, 2009, New Orleans, LA
www.asbmb.org
- ✓ **Microanalysis of Particles**
April 21-23, 2009, Westmont, IL
www.microbeamanalysis.org/meetings/topical/Particles2009/index.htm
- ✓ **Analytical and Quantitative Light Microscopy**
May 6-15, 2009, MBL, Woods Hole, MA
www.mbl.edu/education/courses/special_topics/aqlm.html
- ✓ **EMBO - Light Microscopy in Living Cells**
May 29-June 5, 2009, Oeiras, Portugal
cwp.embo.org/pc09-24/index.html
- ✓ **Lehigh Microscopy School (Multiple Courses)**
June 1-13, 2009, Bethlehem, PA
www.lehigh.edu/microscopy/
- ✓ **Frontiers in Polymer Science**
June 7-9, 2009, Mainz, Germany
www.frontiersinpolymerscience.com
- ✓ **14th Short Course on 3D Microscopy of Living Cells**
June 13-25, 2009, Vancouver, BC, Canada
www.3dcourse.ubc.ca/
- ✓ **Basic Confocal Microscopy Workshop**
June 15-19, 2009, Columbia, SC
dba.med.sc.edu/irf/price/irf/irf.htm
- ✓ **36th MSC Annual Meeting**
June 17-19, 2009, Winnipeg, Canada
msc.rsvs.ulaval.ca
- ✓ **Inter/Micro Conference**
July 6-10, 2009, Chicago, IL
www.mcri.org
- ✓ **Microscopy and Microanalysis 2009**
July 26-30, 2009, Richmond, VA
www.msa.microscopy.org
- ✓ **Neuroscience 2009**
October 17-21, 2009, Chicago, IL
www.sfn.org

2010

- ✓ **Microscopy and Microanalysis 2010**
August 1-5, 2010, Portland, OR

2011

- ✓ **Microscopy and Microanalysis 2011**
August 7-11, 2011, Nashville, TN

2012

- ✓ **Microscopy and Microanalysis 2012**
July 29-August 2, Phoenix, AZ

Please check the "Calendar of Meetings and Courses" in the MSA journal "Microscopy and Microanalysis" for more details and a much larger listing of meetings and courses.

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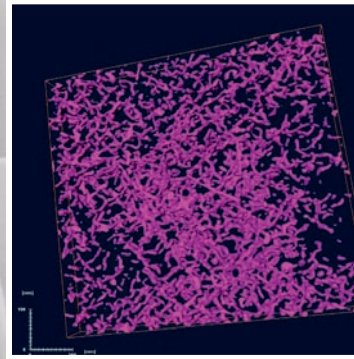
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Solutions for applied Materials Research

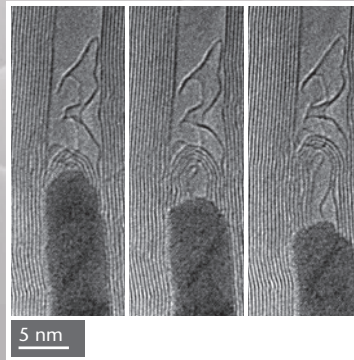
3D NanoCharacterization discover at the nanoscale



Electron tomography enables 3D visualization of nanonetworks.

Courtesy of Dr. Joachim Loos, Eindhoven University of Technology, Netherlands.

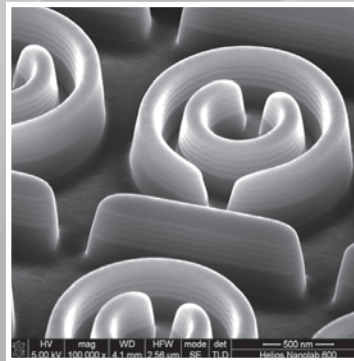
In Situ NanoProcesses experiment down to the atomic scale



Materials confined within nanotubes potentially provide an in situ atomic scale chemical reaction chamber in the TEM.

Courtesy of Julio A. Rodriguez-Mano, Florian Banhart and Mauricio Terrones, IPICYT, Mexico.

3D NanoPrototyping create down to the nanoscale



Split-ring resonator array with a critical dimension of 120nm, prepared by FIB direct.

Background image: Split-ring resonator array with a critical dimension of 120nm, prepared by FIB direct. Image is darkened for artistic impression.